SEQUENCE LISTING

<110> Reiter, Robert E.
      Witte, Owen N.

<120> PSCA: PROSTATE STEM CELL ANTIGEN

<130> 30435.54USU1

<140> 09/038,261
<141> 1998-03-10

<150> 08/814,279
<151> 1997-03-10

<150> 60/071,141
<151> 1998-01-12

<150> 60/074,675
<151> 1998-02-13

<160> 15

<170> PatentIn Ver. 2.0

<210> 1
<211> 998
<212> DNA
<213> HUMAN PSCA (hPSCA)

<220>
<221> misc_feature
<222> (543)
<223> any nucleotide (i.e. a, c, g or t)

<220>
<221> misc_feature
<222> (580)
<223> any nucleotide (i.e. a, c, g or t)

<220>
<221> misc_feature
<222> (584)
<223> any nucleotide (i.e. a, c, g or t)

<220>
<221> misc_feature
<222> (604)

45

<223> any nucleotide (i.e. a, c, g or t)

<220>
<221> misc_feature
<222> (608)
<223> any nucleotide (i.e. a, c, g or t)

<220>
<221> misc_feature
<222> (615)
<223> any nucleotide (i.e. a, c, g, or t)

<220>
<221> misc_feature
<222> (636)
<223> any nucleotide (i.e. a, c, g, or t)

<220>
<221> misc_feature
<222> (640)
<223> any nucleotide (i.e. a, c, g or t)

<220>
<221> misc_feature
<222> (646)
<223> any nucleotide (i.e. a, c, g, or t)

<220>
<221> misc_feature
<222> (697)
<223> any nucleotide (i.e. a, c, g or t)

<220>
<221> misc_feature
<222> (926)
<223> any nucleotide (i.e. a, c, g or t)

<400> 1
agggagaggc agtgaccatg aaggctgtgc tgcttgccct gttgatggca ggcttggccc 60
tgcagccagg cactgccctg ctgtgctact cctgcaaagc ccaggtgagc aacgaggact 120
gcctgcaggt ggagaactgc acccagctgg gggagcagtg ctggaccgcg cgcatccgcg 180
cagttggcct cctgaccgtc atcagcaaag gctgcagctt gaactgcgtg gatgactcac 240
aggactacta cgtgggcaag aagaacatca cgtgctgtga caccgacttg tgcaacgcca 300
gcggggccca tgccctgcag ccggctgccg ccatccttgc gctgctccct gcactcggcc 360
tgctgctctg gggacccggc cagctatagg ctctgggggg ccccgctgca gcccacactg 420
ggtgtggtgc cccaggcctt tgtgccactc ctcacagaac ctggcccagt gggagcctgt 480
cctggttcct gaggcacatc ctaacgcaag tttgaccatg tatgtttgca ccccttttcc 540
ccnaacccctg accttcccat gggccttttc caggattccn accnggcaga tcagtttttag 600

tganacanat ccgcntgcag atggcccctc caaccntttn tgttgntgtt tccatggccc 660
agcattttcc acccttaacc ctgtgttcag gcacttnttc ccccaggaag ccttccctgc 720
ccaccccatt tatgaattga gccaggtttg gtccgtggtg tcccccgcac ccagcagggg 780
acaggcaatc aggagggccc agtaaaggct gagatgaagt ggactgagta gaactggagg 840
acaagagttg acgtgagttc ctgggagttt ccagagatgg ggcctggagg cctggaggaa 900
ggggccaggc ctcacatttg tggggntccc gaatggcagc ctgagcacag cgtaggccct 960
taataaacac ctgttggata agccaaaaaa aaaaaaaa   998

<210> 2
<211> 123
<212> PRT
<213> HUMAN PSCA (hPSCA)

<220>
<221> SITE
<222> (50)..(64)

<220>
<221> SITE
<222> (71)..(82)

<400> 2
Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly Leu Ala Leu Gln
1   5   10   15

Pro Gly Thr Ala Leu Leu Cys Tyr Ser Cys Lys Ala Gln Val Ser Asn
            20   25   30

Glu Asp Cys Leu Gln Val Glu Asn Cys Thr Gln Leu Gly Glu Gln Cys
        35   40   45

Trp Thr Ala Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile Ser Lys
    50   55   60

Gly Cys Ser Leu Asn Cys Val Asp Asp Ser Gln Asp Tyr Tyr Val Gly
65   70   75   80

Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp Leu Cys Asn Ala Ser Gly
                85   90   95

Ala His Ala Leu Gln Pro Ala Ala Ala Ile Leu Ala Leu Leu Pro Ala
            100   105   110

Leu Gly Leu Leu Leu Trp Gly Pro Gly Gln Leu
            115   120

<210> 3

<211> 441
<212> DNA
<213> MURINE PSCA (mPSCA)

<400> 3
atgaagacag ttttttttat cctgctggcc acctacttag ccctgcatcc aggtgctgct 60
ctgcagtgct attcatgcac agcacagatg aacaacagag actgtctgaa tgtacagaac 120
tgcagcctgg accagcacag ttgctttaca tcgcgcatcc gggccattgg actcgtgaca 180
gttatcagta agggctgcag ctcacagtgt gaggatgact cggagaacta ctatttgggc 240
aagaagaaca tcacgtgctg ctactctgac ctgtgcaatg tcaacggggc ccacaccctg 300
aagccaccca ccaccctggg gctgctgacc gtgctctgca gcctgttgct gtggggctcc 360
agccgtctgt aggctctggg agagcctacc atagcccgat tgtgaaggga tgagctgcac 420
tccaccccac ccccacacag g   441

<210> 4
<211> 123
<212> PRT
<213> MURINE PSCA (mPSCA)

<400> 4
Met Lys Thr Val Phe Phe Ile Leu Leu Ala Thr Tyr Leu Ala Leu His
 1   5   10   15

Pro Gly Ala Ala Leu Gln Cys Tyr Ser Cys Thr Ala Gln Met Asn Asn
                20   25   30

Arg Asp Cys Leu Asn Val Gln Asn Cys Ser Leu Asp Gln His Ser Cys
            35   40   45

Phe Thr Ser Arg Ile Arg Ala Ile Gly Leu Val Thr Val Ile Ser Lys
        50   55   60

Gly Cys Ser Ser Gln Cys Glu Asp Asp Ser Glu Asn Tyr Tyr Leu Gly
 65   70   75   80

Lys Lys Asn Ile Thr Cys Cys Tyr Ser Asp Leu Cys Asn Val Asn Gly
                85   90   95

Ala His Thr Leu Lys Pro Pro Thr Thr Leu Gly Leu Leu Thr Val Leu
                100   105   110

Cys Ser Leu Leu Leu Trp Gly Ser Ser Arg Leu
            115   120

<210> 5
<211> 131
<212> PRT

<213> HUMAN STEM CELL ANTIGEN-2 (hSCA-2)

<400> 5
```
Met Lys Ile Phe Leu Pro Val Leu Leu Ala Ala Leu Leu Gly Val Glu
 1               5                  10                  15

Arg Ala Ser Ser Leu Met Cys Phe Ser Cys Leu Asn Gln Lys Ser Asn
            20                  25                  30

Leu Tyr Cys Leu Lys Pro Thr Ile Cys Ser Asp Gln Asp Asn Tyr Cys
        35                  40                  45

Val Thr Val Ser Ala Ser Ala Gly Ile Gly Asn Leu Val Thr Phe Gly
        50                  55                  60

His Ser Leu Ser Lys Thr Cys Ser Pro Ala Cys Pro Ile Pro Glu Gly
 65                  70                  75                  80

Val Asn Val Gly Val Ala Ser Met Gly Ile Ser Cys Cys Gln Ser Phe
                85                  90                  95

Leu Cys Asn Phe Ser Ala Ala Asp Gly Gly Leu Arg Ala Ser Val Thr
                100                 105                 110

Leu Leu Gly Ala Gly Leu Leu Leu Ser Leu Leu Pro Ala Leu Leu Arg
        115                 120                 125

Phe Gly Pro
        130
```

<210> 6
<211> 123
<212> PRT
<213> HUMAN PSCA (hPSCA)

<400> 6
```
Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly Leu Ala Leu Gln
 1               5                  10                  15

Pro Gly Thr Ala Leu Leu Cys Tyr Ser Cys Lys Ala Gln Val Ser Asn
            20                  25                  30

Glu Asp Cys Leu Gln Val Glu Asn Cys Thr Gln Leu Gly Glu Gln Cys
        35                  40                  45

Trp Thr Ala Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile Ser Lys
        50                  55                  60
```

Gly Cys Ser Leu Asn Cys Val Asp Asp Ser Gln Asp Tyr Tyr Val Gly
65   70   75   80

Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp Leu Cys Asn Ala Ser Gly
                85   90   95

Ala His Ala Leu Gln Pro Ala Ala Ala Ile Leu Ala Leu Leu Pro Ala
                100   105   110

Leu Gly Leu Leu Leu Trp Gly Pro Gly Gln Leu
                115   120

<210> 7
<211> 123
<212> PRT
<213> MURINE PSCA (mPSCA)

<400> 7
Met Lys Thr Val Leu Phe Leu Leu Leu Ala Thr Tyr Leu Ala Leu His
    1   5   10   15

Pro Gly Ala Ala Leu Gln Cys Tyr Ser Cys Thr Ala Gln Met Asn Asn
                20   25   30

Arg Asp Cys Leu Asn Val Gln Asn Cys Ser Leu Asp Gln His Ser Cys
            35   40   45

Phe Thr Ser Arg Ile Arg Ala Ile Gly Leu Val Thr Val Ile Ser Lys
        50   55   60

Gly Cys Ser Ser Gln Cys Glu Asp Asp Ser Glu Asn Tyr Tyr Leu Gly
65   70   75   80

Lys Lys Asn Ile Thr Cys Cys Tyr Ser Asp Leu Cys Asn Val Asn Gly
                85   90   95

Ala His Thr Leu Lys Pro Pro Thr Thr Leu Gly Leu Leu Thr Val Leu
                100   105   110

Cys Ser Leu Leu Leu Trp Gly Ser Ser Arg Leu
                115   120

<210> 8
<211> 15
<212> PRT

<213> HUMAN PSCA (hPSCA)

<400> 8
Thr Ala Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile Ser Lys
 1   5   10   15

<210> 9
<211> 12
<212> PRT
<213> HUMAN PSCA (hPSCA)

<400> 9
Val Asp Asp Ser Gln Asp Tyr Tyr Val Gly Lys Lys
 1   5   10

<210> 10
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: RT-PCR PRIMER

<400> 10
ttctcctgct ggccacctac   20

<210> 11
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: RT-PCR PRIMER

<400> 11
gcagctcatc ccttcacaat   20

<210> 12
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: RT-PCR PRIMER

<400> 12

```
tgcttgccct gttgatggca g                                            21


<210> 13
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: RT-PCR PRIMER

<400> 13
ccagagcagc aggccgagtg ca                                           22


<210> 14
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: RT-PCR PRIMER

<400> 14
ggagaattca tggcactgcc ctgctgtgct ac                                32


<210> 15
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: RT-PCR PRIMER

<400> 15
ggagaattcc taatgggccc cgctggcgtt                                   30
```

Please amend the subject application as follows:

In the claims:

Please cancel claim 48 without prejudice to pursue the subject matter of these claims in a related application. Please enter new claim 49.

--49. (new)   A method for selectively killing a cell expressing Prostate Stem Cell Antigen (PSCA) comprising reacting a monoclonal antibody designated ATCC No. HB-12612, ATCC No. HB-12616, ATCC No. HB12618, or ATCC No. HB-12617 conjugated to a therapeutic agent with the cell so that the therapeutic agent so conjugated can kill the cell expressing PSCA.--

In the Specification:

At page 4, lines 21-22, please delete "FIG. 1. Nucleotide (A) and translated amino acid (B) sequences of a cDNA encoding human PSCA (ATCC Designation 209612)" and insert

--FIG. 1A. Nucleotide sequences of a cDNA encoding human PSCA (ATCC Designation 209612).

Fig. 1B. Translated amino acid sequences of a cDNA encoding human PSCA (ATCC Designation 209612).--

At page 5, lines 3-11, please delete "FIG. 7. Restricted Expression of PSCA mRNA in normal and cancerous tissues. A: RT-PCR analysis of PSCA expression in normal human tissues demonstrating high expression in prostate, placenta, and tonsils. 1ng of reverse-transcribed first strand cDNA (Clontech, Palo Alto, CA) from the indicated tissues was

amplified with PSCA gene specific primers. Data shown are from 30 cycles of amplification. **B:** RT-PCR analysis of PSCA expression demonstrating high level in prostate cancer xenografts and normal tissue. 5ng of reverse-transcribed cDNA from the indicated tissues was amplified with PSCA gene specific primers. Amplification with β-actin gene specific primers demonstrate normalization of the first strand cDNA of the various samples. Data shown are from 25 cycles of amplification. AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line" and insert

--**FIG. 7A.** Restricted Expression of PSCA mRNA in normal and cancerous tissues. RT-PCR analysis of PSCA expression in normal human tissues demonstrating high expression in prostate, placenta, and tonsils. 1ng of reverse-transcribed first strand cDNA (Clontech, Palo Alto, CA) from the indicated tissues was amplified with PSCA gene specific primers. Data shown are from 30 cycles of amplification.

FIG. 7B. Restricted Expression of PSCA mRNA in normal and cancerous tissues. RT-PCR analysis of PSCA expression demonstrating high level in prostate cancer xenografts and normal tissue. 5ng of reverse-transcribed cDNA from the indicated tissues was amplified with PSCA gene specific primers. Amplification with β-actin gene specific primers demonstrate normalization of the first strand cDNA of the various samples. Data shown are from 25 cycles of amplification. AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.--

At page 5, line 13, please delete "FIG. 8. Schematic representation of human PSCA, murine PSCA, and human Thy-1/Ly-6 gene structures" and insert

--**FIG. 8A.** Schematic representation of human Thy-1/Ly-6 gene structures.

FIG. 8B. Schematic representation of murine PSCA gene structure.

FIG. 8C. Schematic representation of human PSCA gene structure. --

At page 5, lines 15-19, please delete "FIG. 9. Northern blot analysis of PSCA expression. A: Total RNA from normal prostate and LAPC-4 androgen dependent (AD) and independent (AI) prostate cancer xenografts were analyzed using PSCA or PSA specific probes. Equivalent RNA loading and RNA integrity were demonstrated separately by ethidium staining for 18S and 28S RNA. B: Human multiple tissue Northern blot analysis of PSCA. The filter was obtained from Clontech (Palo Alto, CA) and contains 2ug of polyA RNA in each lane" and insert

-- **FIG. 9A.** Northern blot analysis of PSCA expression. Total RNA from normal prostate and LAPC-4 androgen dependent (AD) and independent (AI) prostate cancer xenografts were analyzed using PSCA or PSA specific probes. Equivalent RNA loading and RNA integrity were demonstrated separately by ethidium staining for 18S and 28S RNA.

FIG. 9B. Northern blot analysis of PSCA expression. Human multiple tissue Northern blot analysis of PSCA. The filter was obtained from Clontech (Palo Alto, CA) and contains 2ug of polyA RNA in each lane. --

At page 5, lines 21-27, please delete "FIG. 10. Northern blot comparison of PSCA, PSMA, and PSA expression in prostate cancer xenografts and tumor cell lines. PSCA and PSMA demonstrate high level prostate cancer specific gene expression. 10 μg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSCA, PSMA, and PSA cDNA fragments. Shown are 4 hour and 72 hour autoradiographic exposures of the membrane and the ethidium bromide gel demonstrating equivalent loading of samples. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line" and insert

-- **FIG. 10-1.** Northern blot analysis of PSCA expression in prostate cancer xenografts and tumor cell lines. PSCA demonstrates high level prostate cancer specific gene expression. 10 µg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSCA cDNA fragments. Shown are 4 hour and 72 hour autoradiogrphic exposures of the membrane. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

FIG. 10-2. Northern blot analysis of PSM expression in prostate cancer xenografts and tumor cell lines. PSM demonstrates high level prostate cancer specific gene expression. 10 µg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSM cDNA fragments. Shown are 4 hour and 72 hour autoradiogrphic exposures of the membrane. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

FIG. 10-3. Northern blot analysis of PSA expression in prostate cancer xenografts and tumor cell lines. 10 µg of total RNA from the indicated tissues were size fractionated on an agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized sequentially with ^{32}P-labelled probes representing PSA cDNA fragments. Shown are 4 hour and 72 hour autoradiogrphic exposures of the membrane and the ethidium bromide gel demonstrating equivalent loading of samples. BPH, benign prostatic hyperplasia; AD, androgen-dependent; AI, androgen-independent; IT, intratibial xenograft; C.L., cell line.

At page 5, lines 29-35, please delete "FIG. 11. In situ hybridization with antisense riboprobe for human PSCA on normal and malignant prostate specimens. A: PSCA is expressed by a subset of basal cells within the basal cell epithelium (black arrows), but

not by the terminally differentiated secretory cells lining the prostatic ducts (400X magnification). B: PSCA is expressed strongly by a high grade prostatic intraepithelial neoplasia (PIN) (black arrow) and by invasive prostate cancer glands (yellow arrows), but is not detectable in normal epithelium (green arrow) at 40X magnification. C: Strong expression of PSCA in a case of high grade carcinoma (200X magnification)" and insert

-- **FIG. 11A.** In situ hybridization with antisense riboprobe for human PSCA on normal prostate specimens. PSCA is expressed by a subset of basal cells within the basal cell epithelium, but not by the terminally differentiated secretory cells lining the prostatic ducts (400X magnification).

FIG. 11B. In situ hybridization with antisense riboprobe for human PSCA on normal and malignant prostate specimens. PSCA is expressed strongly by a high grade prostatic intraepithelial neoplasia (PIN) and by invasive prostate cancer glands, but is not detectable in normal epithelium at 40X magnification.

FIG. 11C. In situ hybridization with antisense riboprobe for human PSCA on malignant prostate specimens. Strong expression of PSCA in a case of high grade carcinoma (200X magnification). --

At page 6, lines 1-9, please delete "FIG. 12. Biochemical analysis of PSCA. A: PSCA was immunoprecipitated from 293T cells transiently transfected with a PSCA construct and then digested with either N-glycosidase F or O-glycosidase, as described in Materials and Methods. B: PSCA was immunoprecipitated from 293T transfected cells, as well as from conditioned media of these cells . Cell-associated PSCA migrates higher than secreted or shed PSCA on a 15% polyacrylamide gel. C:FACS analysis of mock-transfected 293T cells, PSCA-transfected 293T cells and LAPC-4 prostate cancer xenograft cells using an affinity purified polyclonal anti-PSCA antibody. Cells were not permeabilized in order to detect only surface expression. The y axis represents relative

cell number and the x axis represents fluorescent staining intensity on a logarithmic

scale" and insert

FIG. 12A. Biochemical analysis of PSCA. PSCA was immunoprecipitated from 293T

cells transiently transfected with a PSCA construct and then digested with either N-

glycosidase F or O-glycosidase, as described in Materials and Methods.

FIG. 12B: Biochemical analysis of PSCA. PSCA was immunoprecipitated from 293T

transfected cells, as well as from conditioned media of these cells . Cell-associated PSCA

migrates higher than secreted or shed PSCA on a 15% polyacrylamide gel.

FIG 12C. Biochemical analysis of PSCA. FACS analysis of mock-transfected 293T

cells, PSCA-transfected 293T cells and LAPC-4 prostate cancer xenograft cells using an

affinity purified polyclonal anti-PSCA antibody. Cells were not permeabilized in order to

detect only surface expression. The y axis represents relative cell number and the x axis

represents fluorescent staining intensity on a logarithmic scale --

At page 6, lines 19-22, please delete "FIG. 14. Flow Cytometric analysis of cell surface

PSCA expression on prostate cancer xenograft (LAPC-9), prostate cancer cell line

(LAPC-4) and normal prostate epithelial cells (PreC) using anti-PSCA monoclonal

antibodies 1G8 (green) and 3E6 (red), mouse anti-PSCA polyclonal serum (blue), or

control secondary antibody (black). See Example 5 for details." and insert

FIG. 14A. Flow Cytometric analysis of cell surface PSCA expression on prostate

cancer xenograft (LAPC-9) using anti-PSCA monoclonal antibodies 1G8 and 3E6, mouse

anti-PSCA polyclonal serum, or control secondary antibody. See Example 5 for details.

FIG. 14B. Flow Cytometric analysis of cell surface PSCA expression on prostate cancer

cell line (LAPC-4) using anti-PSCA monoclonal antibodies 1G8 and 3E6, mouse anti-

PSCA polyclonal serum, or control secondary antibody. See Example 5 for details.

FIG 14C. Flow Cytometric analysis of cell surface PSCA expression on normal prostate epithelial cells (PreC) using anti-PSCA monoclonal antibodies 1G8 and 3E6, mouse anti-PSCA polyclonal serum, or control secondary antibody. See Example 5 for details.

At page 6, line 24, please delete "FIG. 15. Epitope mapping of anti-PSCA monoclonal antibodies 1G8 and 3E6. See Example 5 for details." and insert

~~FIG. 15A. An epitope map for each of the seven disclosed antibodies.~~

FIG 15A. Epitope mapping of anti-PSCA monoclonal antibodies conducted by Western blot analysis of GST-PSCA fusion proteins.

REMARKS

Claims 44-48 were pending. Claim 48 has been cancelled herein. New claim 49 is submitted herein.

New claim 49 is supported in the specification at page 12, lines 14-16; page 13, lines 1-7; page 13, lines 33-36; page 14, lines 1-18; page 23, lines 20-25; page 23, lines 29-35; and page 24, lines 1-11.

New claim 49 is supported by the specification as originally filed and do not involve new matter. Accordingly, applicants respectfully request entry of claim 49.

The changes to the specification, at pages 4-6, are to provide separate descriptions of the figures that contain multiple sub-figures. The changes do not involve new matter. Entry of these changes is requested.